Molecular Basis of Oculocutaneous Albinism

William S. Oetting* and Richard A. King*†‡
*Departments of Medicine, †Pediatrics, and ‡The Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota, U.S.A.

Oculocutaneous albinism (OCA) is a complex group of genetic disorders that have historically been defined by clinical and biochemical methods. Recent advances in the molecular biology of pigmentation have greatly increased our understanding of the complexity of this group of disorders. To date, two different types of OCA (OCA1 and OCA2) have been mapped to specific chromosomal regions. Mutations have been found in the tyrosinase locus associated with OCA1 and the human homologue to the murine pink-eyed dilution locus associated with OCA2. Analysis of these genes and their mutations will allow us to better define and categorize the different types of albinism. Further, the analysis of these genes and their mutations will provide information on the role of these gene products in melanin biosynthesis and the effect specific mutations have on the pathogenesis of albinism. ***J Invest Dermatol 103:131S–136S, 1994***

DEFINITION OF ALBINISM

Albinism represents a group of inherited abnormalities of melanin synthesis characterized as a congenital reduction or absence of melanin synthesis in association with specific ocular changes resulting from hypopigmentation in the developing eye [1,2]. These ocular changes include foveal hypoplasia, misrouting of the optic fibers as they course from the retina to the brain, and a reduction in visual acuity. Unlike the cutaneous pigmentation, which is variable with the different mutant alleles that are associated with albinism, the changes in the optic system are universal for all types of albinism and are a major part of the diagnostic criteria [3,4]. The color of the hair and skin or the presence or absence of the ability to tan are no longer sufficient for definition or recognition of albinism.

Albinism has been broadly divided into two types, ocular albinism (OA) with primary involvement of the eye, and oculocutaneous albinism (OCA) with involvement of the skin, hair and the eye. OCA can be further divided into OCA1 or tyrosinase related OCA, OCA2 or tyrosinase positive OCA, unclassified types (brown OCA, red OCA), and several types for which the primary defect is not limited to the melanocyte (**Table I**). OA presents as an X-linked recessive OA (OA1), associated with macromelanosomes in the melanocytes, or as autosomal recessive OA. At present, three genetic loci involved in albinism have been mapped to specific chromosomal regions (OCA1, OCA2 and OA1) and two of these genes have been isolated and mutations responsible for the OCA have been identified (OCA1 and OCA2). This review will address OCA.

TYROSINASE RELATED OCULOCUTANEOUS ALBINISM: OCA1

Tyrosinase related OCA (OCA1) is the result of the loss of function of tyrosinase in melanocytes because of mutations of the tyrosinase gene. There are several types of OCA1, each having a somewhat unique phenotype (**Table 1**). OCA1A or tyrosinase negative OCA has a totally depigmented phenotype [1,2]. Affected individuals have white hair and skin and blue irides throughout their life. All skin lesions are amelanotic. OCA1B or yellow OCA has a variably pigmented phenotype [5,6]. Affected individuals have white hair at birth and develop pigmented hair, yellow or blond, in the first decade of life. The skin remains white but may tan with sun exposure and pigmented nevi and freckles may develop. OCA1MP or minimal pigment OCA is similar to OCA1B except that less pigment is apparent and is usually localized to the iris with little change in hair or skin color [7]. Finally, OCA1TS or temperature sensitive OCA has a pigmentation phenotype characterized by the formation of melanin in hair located in cooler regions (arms and legs) of the body in a pattern similar to the Siamese cat [8,9]. As noted above, the cutaneous and even the iris pigment can be absent or moderate in amount in the different types of OCA1, but the ocular features are constant.

An important distinguishing characteristic of OCA1 is the presence of marked hypopigmentation at birth. Most individuals affected with OCA1 have white hair, milky white skin, and blue eyes at birth. During the first and second decade of life, the irides usually become a darker blue or blue-gray and the skin may appear to have more color, even in OCA1A. Sun exposure produces erythema and a burn if the skin is unprotected and a generalized tan is absent or minimal in most individuals with OCA1. Few pigmented lesions (nevi, freckles, and letigines) develop in the skin.

The locus for OCA1 has been mapped to the tyrosinase locus (TYR) at chromosome 11q14-21 and mutations in the tyrosinase gene are the cause of this genetic condition [10,11]. A series of TYR mutant alleles have been identified, associated with a range of residual enzyme activity. Some alleles are associated with no enzyme function while others have residual or unusual activity. The recognized phenotype can be explained by the effect of the mutation on tyrosinase activity, and this has allowed previously unclassified OCA types to be identified as OCA1.

TY OSINASE GENE MUTATIONS ASSOCIATED WITH OCA1

We are currently using a combination of methods to detect mutations responsible for OCA1 including quantitation of tyrosinase activity, single-stranded conformational polymorphism analysis (PCR-SSCP) and fluorescence DNA sequencing. Analysis of the levels of tyrosinase activity is important to determine the type of OCA involved. Quantitation of tyrosinase activity is accomplished using the tritiated tyrosinase assay with hairbulb melanocytes as the

Reprint requests to: Dr. Richard A. King, Department of Medicine, Genetics, Box 485 U.M.H.C., 420 Delaware St. S.E., University of Minnesota, Minneapolis, MN 55455.

0022-202X/94/$07.00

Table I. Classification of Oculocutaneous Albinism (OCA)

Primary abnormality of melanin synthesis in the melanocyte
OCA1; Tyrosinase Related OCA
Mutations associated with no residual enzyme activity
OCA1A: tyrosinase-negative OCA
Mutations associated with residual enzyme activity
OCA1B: yellow OCA
OCA1MP: minimal pigment OCA
Mutations associated with unusual enzyme activity
OCA1TS: temperature-sensitive OCA
OCA2; tyrosinase positive OCA, Prader Willi/Angelman Syndrome
Unclassified types
Brown OCA
Rufous or red OCA
Abnormality not limited to the melanocyte
Hermansky-Pudlak syndrome
Chediak-Higashi syndrome

source of enzyme [12,13]. Reduced or absent levels of tyrosinase activity is one of the criteria for OCA1. Most obligate heterozygotes for OCA1 have low hairbulb tyrosinase activity, but recent studies show that some mutant alleles produce sufficient residual activity to give carriers normal or near normal levels of hairbulb tyrosinase activity. If tyrosinase activity levels of the affected individual indicate that OCA1 is the type of albinism involved, then their tyrosinase gene is analyzed for mutations.

For analysis of the tyrosinase gene, each of the five exons are first screened for mutations using PCR-SSCP [14–16]. This method provides detection of point mutations in DNA by observing mobility shifts in single stranded DNA on a non-denaturing polyacrylamide gel that results from the changes in the nucleotide sequence [15]. Each exon is individually amplified using the polymerase chain reaction (PCR), denatured, and the strands separated by electrophoresis. Amplification primers hybridize to the flanking intron sequences allowing the entire coding region and the intron/exon boundaries to be analyzed. When an exon exhibits altered electrophoretic mobility, alterations in the sequence are determined using automated fluorescence infrared DNA sequencing [16].

The amplified exon used in PCR-SSCP analysis is also used as the template for di-deoxy sequencing employing a cycle-sequencing protocol [16]. The sequencing primers are fluorescently tagged with fluors that emit in the infrared region of the spectrum. The sequencing ladder is analyzed using an automated fluorescence DNA sequencer (LI-COR model 4000). The resulting nucleotide sequence is then compared to the normal tyrosinase sequence and changes noted. The combination of PCR-SSCP with fluorescent di-deoxy DNA sequencing allows for the fast and accurate detection of mutations in the proband and for the determination of the carrier status of relatives. The advantages to this system are the elimination of radioisotopes for detection of both the PCR-SSCP analysis and sequencing and the quickness and ease of these techniques.

A large number of mutations of the tyrosinase gene have been identified (**Table II**) [17,18]. Most are single-base substitutions that produce an amino acid substitution in the protein. Less frequent are base substitutions that create a stop codon and one-, two- or four-base insertions or deletions that alter the reading frame and produce a truncated protein. More recently, a number of single-base substitutions that alter a splice donor or acceptor site have been identified.

Most individuals with OCA1 in the U.S. population have been compound heterozygotes with a different maternal and paternal mutation. Individuals who are homozygous for a single mutation have often come from small populations or from consanguineous matings. In approximately 15% of the individuals who are compound heterozygotes, only one of the two mutant alleles has been identified. In these cases, the individual is heterozygous for this mutation, showing that a second mutation must be present. Possibilities for the second mutation are cryptic splice sites, gross genomic rearrangements undetected by sequencing, or mutations in the 5′ promoter site. Evidence has been accumulating that there are important regulatory regions several kilobases 5′ of the promoter region. Different analytical methods will be needed to detect these different possibilities.

The majority of mutations identified to date produce a totally inactive enzyme or no enzyme protein, and these are associated with OCA1A, and with a total life-long inability to synthesize melanin in any melanocyte. There has been a strong bias in selecting individuals with OCA1A for analysis because this phenotype is the most obvious clinically. A small number of mutations have been found with OCA1MP and OCA1B, both pigmenting types of OCA. It is hypothesized that these mutations produce enzymes with some residual activity, accounting for the melanin synthesis. The yellow or blond hair color of OCA1B is thought to be the result of small amounts of DOPAquinone being made in the initial steps of the melanin pathway, followed by the rapid reaction of this compound with sulfhydryl compounds to form cysteinylDOPA and eventually pheomelanin.

OCA1B is quite variable in degree of hypopigmentation, varying from very little pigment associated with whitish-blond hair to nearly normal pigment with dark blond hair and skin that tans well. The variability will likely be the result of the amount of residual tyrosinase activity [6] and the genetic pigment constitution of the affected individual. Some mutant alleles are associated with sufficient residual activity to produce moderate amounts of cutaneous pigment, but all mutant alleles are associated with insufficient ocular pigment for normal foveal and optic tract development. One mutation has been found with OCA1TS or temperature sensitive OCA, and the activity of the mutant tyrosinase enzyme was temperature sensitive, with reduced activity above 35°C [8,9].

Further genetic complexity comes from the fact that most affected individuals are compound heterozygotes. As a result, an individual may have two different mutant alleles that code for inactive enzyme, one mutant allele coding for inactive enzyme and one mutant allele coding for enzyme with residual activity, or two different mutant alleles that code for enzyme with residual activity. Clinical and biochemical analysis will not elucidate this type of complexity, and molecular analysis is the only accurate method of specific diagnosis.

ANALYSIS OF THE TYROSINASE MUTATIONS

Tyrosinase (EC 1.14.18.1) is a bicupric enzyme responsible for the first two steps in the melanin-synthesis pathway; the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone [19]. The first reaction, tyrosine to DOPA, requires the presence of molecular oxygen, which first forms a peroxide with the two copper atoms. This complex compound then reacts with the 3 or 5 carbon atom of tyrosine resulting in the addition of a hydroxyl group forming DOPA. The two hydroxyl groups are then oxidized to produce DOPAquinone. It has also been hypothesized that the formation of DOPAquinone from tyrosine occurs as a single step resulting from a ligand substitution between the bound dioxygen with the newly formed diphenol which is then subsequently released as DOPAquinone [20,21]. A third activity, 5,6-dihydroxyindole (DHI) oxidase has also been ascribed to tyrosinase but the biologic role of this activity is unknown [22,23].

Analysis of the mutations of the tyrosinase gene in individuals with OCA1 has revealed that the identified missense mutations cluster into four regions of the tyrosinase gene (**Fig 1**) [24]. It is felt that these clusters define important functional regions of tyrosinase involved in the catalytic activity of the enzyme. Two of these regions are the copper A and copper B binding sites containing codons 172-223 for copper A and 352-401 for copper B. The missense mutations found in the two copper binding sites are listed in **Table III** [17,25]. Each copper binding site consists of two α-helical regions and a loop structure between the two α-helices. The purpose of the two α-helices is to orient the three histidine residues so they can complex with a copper atom. The two copper binding sites bring the two copper atoms to a distance of 3.6 Å as observed in

Table II. Mutations and Polymorphisms Associated with OCA1

Mutation	Base Change(s)	Base Change	Type OCA1	Population
P21S[b]	CCT → TCT	Pro → Ser	A	Caucasians
D42G	GAC → GGC	Asp → Gly	A	Caucasians
G47D	GGC → GAC	Gly → Asp	A	Caucasians and Hispanics
C55Y	TGT → TAT	Cys → Tyr	A	Caucasians
R77Q	CGG → CAG	Arg → Gln	A	Japanese
P81L	CCT → CTT	Pro → Leu	A	Caucasians
C89R	TGC → CGC	Cys → Arg	A	Blacks
+ΔA96	ATG[b] → AATG	Term 168	A	Caucasians
−ΔGA115	AGA → A– –	Term 167	A	Pakistani
F176I	TTT → ATT	Phe → Ile	A	Caucasians
W178X	TGG → TAG	Trp → Ter	A	Afghans
−ΔG191	GGA → –GA	Term 225	A	Caucasians
Y192S	TAT → TCT	Tyr → Ser	Poly[a]	All except Orientals
A206T	GCT → ACT	Ala → Thr	A	Caucasians
L216M	TTG → ATG	Leu → Met	A	Hispanics
R217W	CGG → TGG	Arg → Trp	A	Caucasians
R217Q	CGG → CAG	Arg → Gln	A	Caucasians
W236X	TGG → TAG	Trp → Ter	A	Blacks
−ΔTG244	TGTG → TG– –	Term 244	A	Caucasians
V275F	GTC → TTC	Val → Phe	B	Caucasians
R299H	CGT → CAT	Arg → His	A	Caucasians
+ΔC310	CCA → CCCA	Term 317	A	Japanese
H367R	CAC → CGC	His → Arg	A	Caucasians
N371T	AAT → ACT	Asn → Thr	A	Caucasians
T373K	ACA → AAA	Thr → Lys	A	Caucasians
Q378X	CAG → TAG	Gln → Ter	A	Caucasians
N382K	AAC → AAA	Asn → Lys	A	Caucasians
D383N	GAT → AAT	Asp → Asn	A	Caucasians
−ΔT388	CTT → CT–	Term 484	A	Caucasians
R402Q	CGA → CAA	Arg → Gln	Poly[a]	All except Orientals
R403S	AGG → AGT	Arg → Ser	B	Caucasians
P406L	CCT → CTT	Pro → Leu	B	Caucasians
G419R	GGA → AGA	Gly → Arg	A	Caucasians
R422Q	CGG → CAG	Arg → Gln	TS	Caucasians
−ΔCTTT438	CTTT → – – – –	Term 484	A	Caucasians
G446S	GGC → AGC	Gly → Ser	A	Caucasians
D448N	GAC → AAC	Asp → Asn	A	Caucasians
Q453X	CAA → TAA	Gln → Ter	A	Caucasians
+ΔT489	ACT → ACTT	Term 509	A	Caucasians
+ΔC501	CGT → CCGT	Term 509	A	Caucasians

[a] Poly, polymorphism.
[b] The start codon ATG (Methionine) is counted as codon 1.

Neurospora tyrosinase, which permits the copper atoms to form a peroxide [26]. Site directed mutagenesis experiments on the *Streptomyces glaucescens* tyrosinase gene has shown the importance of the histidine residues on tyrosinase activity and as copper ligands [27,28].

Comparison of the amino acid sequences between the copper binding sites of several tyrosinase and hemocyanin polypeptides shows that these regions are highly conserved, including two identifiable amino acid motifs [29]. Missense mutations in the copper A binding region can be found within both of these motifs. The A206T mutation affects a motif found in both copper-binding regions (Pro-X-Phe-X-X-X-His) substituting a threonine for an alanine located between the proline and phenylalanine residues. The mutation F1761 in the putative copper A binding region affects a phenylalanine in the motifPhe-X-X-X-His. The phenylalanines in both motifs are thought to be important in the correct positioning of the histidine ligands. The other three mutations are within the α-helix next to the codon 211 histidine ligand.

We have previously reported on the effect of mutations in the

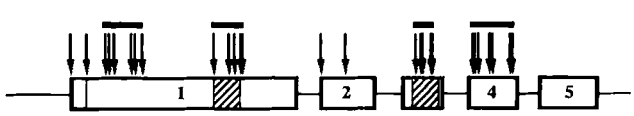

Figure 1. Clustering of the missense mutations of the tyrosinase gene. The five *boxes* represent the exons of the tyrosinase gene. The *striped regions* represent the two putative copper binding regions and the shaded region is the signal peptide. The *arrows* show the location of the missense mutations. The *horizontal bars* represent putative functional domains of the tyrosinase enzyme.

Table III. Missense Mutations in the Tyrosinase Copper Binding Regions

Mutation	Base Change	Codon Change
Copper (A)		
F176I	TTT → ATT	Phe → Ile
A206T	GCT → ACT	Ala → Thr
L216M	TTG → ATG	Leu → Met
R217W	CGG → TGG	Arg → Trp
R217Q	CGG → CAG	Arg → Gln
Copper (B)		
H367R	CAC → CGC	His → Arg
N371T	AAT → ACT	Asn → Thr
T373K	ACA → AAA	Thr → Lys
N382K	AAC → AAA	Asn → Lys
D383N	GAT → AAT	Asp → Asn

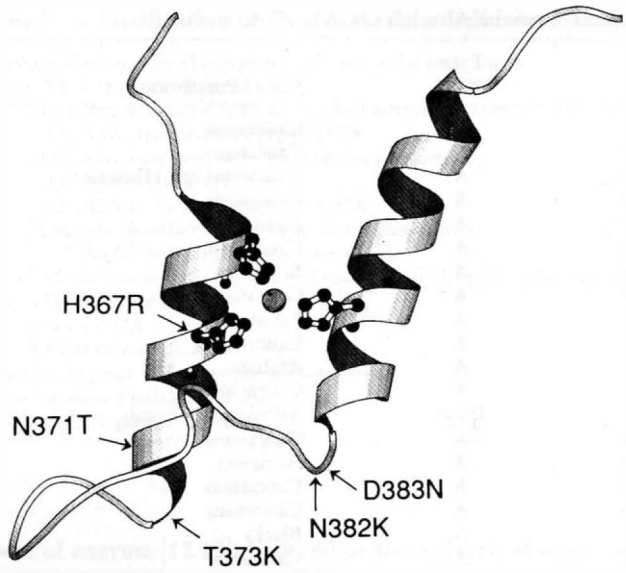

Figure 2. Structure of the copper B binding region of Panulirus interruptus hemocyanin. The copper B atom is the single atom in the center of the figure. The three histidine ligands held in place by the two α-helices are represented as ball-and-stick figures. The α-helices are the two coiled structures. The *arrows* show the homologous codon location of the human tyrosinase mutations associated with OCA1. This image was constructed from the three-dimensional coordinates of *Panulirus interruptus* hemocyanin using the MOLSCRIPT software [30,31,33].

copper B binding region on the secondary structure of tyrosinase [25]. We have done further analysis of this region using the known three-dimensional structure of the arthropod hemocyanin of *Panulirus interruptus* [30,31]. Comparisons between tyrosinase and hemocyanin for both the primary structure and the predicted secondary structure show that they are very similar within the copper B-binding region [25,32]. This makes hemocyanin a good model to study how mutations might affect this same region in tyrosinase. **Figure 2** shows the folding structure of the copper B binding region of hemocyanin [33]. As can be seen in this model, the two α-helices, connected by a loop region, hold the three histidine ligands in place. Four mutations, N371T, T373K, N382K and D383N, are at or near the junction of the α-helix and the loop region *(arrows)*. Computer analysis of the secondary structure of the loop region shows that the incorporation of these mutations into the primary amino acid sequence will alter this structure, when compared to the normal amino acid sequence [25]. Most likely these mutations affect the orientation of the α-helix, which in turn will either affect the location or the binding of the copper atom by the histidine ligands. The copper to copper distance is critical for enzymatic activity and any alteration of this distance would render the enzyme inactive, due to its inability to form a peroxide with molecular oxygen.

A fifth mutation was found at codon 367 (H367R), which substitutes an arginine for a histidine residue (**Fig 3**). This histidine is one of the three copper ligands in the copper B-binding region. As was shown in the site-directed mutagenesis analysis of the *Streptomyces glaucescens* tyrosinase gene, elimination of one of these histidines will result in an inactive enzyme [28]. The chemistry and size of the arginine residue is such that it would not be able to complex with the copper atom and the other two histidine ligands alone are not sufficient to bind the copper atom in the correct orientation relative to the copper A atom [28].

There are two other clusters, one in exon 4 next to the copper B cluster and one at the amino terminal end of the polypeptide (**Fig 1**). The cluster in exon 4 contains mutations that have residual activity (R403S, P406L) and the temperature-sensitive mutation (R422Q). This cluster is close to the active site because of its proximity to the copper B binding region and would most likely be involved in substrate binding. The binding of tyrosine and DOPA must occur next to the copper atoms and the substitution of a single amino acid side chain in this region would be enough to prevent substrate binding. The residual activity mutations may restrict but not abolish substrate binding so that some activity is present.

Analysis of the amino-terminal end of hemocyanin shows that this region of the polypeptide interacts with the two copper-binding sites. This may also be the case in tyrosinase and could explain the amino terminal cluster of mutations. The amino terminal end of the tyrosinase molecule may play a role in forming the substrate-binding site and mutations in this cluster could affect activity by altering the structure of the catalytic site of the enzyme. It has also been hypothesized that there may be two active sites, one responsible for DOPA oxidase and DHI oxidase activity and one responsible for tyrosine hydroxylase activity [23]. If this is true, then the amino-terminal cluster and the exon 4 cluster may represent mutations that are affecting these two different active sites.

These mutations are revealing the complex chemistry of melanin synthesis by tyrosinase. As other mutations are found and their effect on the catalytic activity of tyrosinase determined, we should have a better understanding of the role tyrosinase plays in melanin formation including how mutations cause the different phenotypes observed.

TYROSINASE POSITIVE OCULOCUTANEOUS ALBINISM: OCA2

The general phenotypic features of tyrosinase positive oculocutaneous albinism or OCA2 include the presence of hair and iris pigment, and the development of localized (nevi, freckles and letigines) skin pigment, often in sun-exposed regions of the skin [2]. The ethnic and constitutional pigment background of an affected individual has a more profound effect on OCA2 than on OCA1.

The phenotype of type OCA2 varies such that the cutaneous hypopigmentation can be subtle in some and obvious in others. Several generalizations about OCA2 can be made. First, individuals with OCA2 usually have pigmented hair at birth, which is in contrast to OCA1. Second, the skin is white but it usually has a creamy rather than a milky color, and the eyes are blue or blue-gray. Third, the amount of pigment in the skin, hair, and eyes generally increases with age but rarely reaches an amount that would be considered normal for an unaffected family member. More importantly, affected individuals from more darkly pigmented populations have clinically apparent hypopigmented skin and hair throughout their life, while those from lightly pigmented populations, particularly those of northern Europe (i.e., Scandinavian), may not appear to

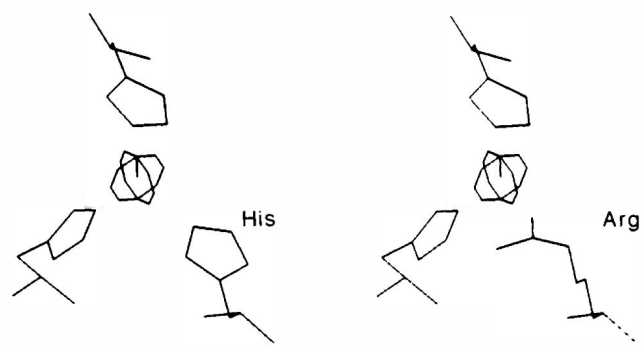

Figure 3. Orientation of the three histidine residues and the copper (B) atom. The figure on the left represents the normal complex and the figure on the right shows the arginine substitution associated with the H367R mutations. This image was constructed from the three-dimensional coordinates of *P. interruptus* hemocyanin using the UCSD Molecular Modeling System (MMS) software [30,31].

have significant cutaneous hypopigmentation associated with the ocular features of albinism. Fourth, hairbulbs from an individual with OCA2 form pigment when incubated in tyrosine or DOPA, and the melanin is synthesized in the melanosomes [34–36].

Caucasian individuals with OCA2 have creamy white skin and may have pigmented birth marks. There is no change in the general skin color with time and a tan does not develop after sun exposure, but localized pigment (i.e., pigmented nevi, freckles) may develop. The hair can be very light at birth, appearing nearly white or light blond, or it can be blond, golden blond, reddish blond, or brown. The iris is blue to blue/gray, and transillumination of the globe often reveals pigment at the pupillary border with strands of pigment coursing through the iris like the spokes of a wagon wheel. With time the amount of hair and iris pigment often increases and the affected individual may not appear to have albinism unless the eyes and skin are carefully examined.

In African-American and African individuals, the phenotype of type OCA2 is distinct, and seems to represent one genetic type [35]. At birth the skin is white, the hair is yellow, and the irides are blue. With time the hair turns a darker yellow and pigmented nevi and lentigines develop in the skin, although there is no tan. The iris color remains blue or turns tan or light brown and there is pigment in the iris at the pupillary border and coursing through the stroma [35].

Hairbulb tyrosinase from individuals with OCA2 has normal activity and a normal electrophoretic pattern [13,36–38], and the defect in this type of OCA is not in the tyrosinase gene. The locus for OCA2 has been mapped to chromosome 15q11.2-q12 and the human homologue of the mouse pink-eyed dilution gene has also been localized to this region and has now been shown to be the gene responsible for OCA2. Molecular analysis of this gene, both its normal function and mutations involved in OCA2 has just begun [39–44].

A newly recognized association with OCA2 is the hypopigmentation found with Prader-Willi syndrome (PWS) and Angelman Syndrome (AS) [45–48]. PWS is a developmental syndrome that includes neonatal hypotonia, hyperphagia, and obesity, hypogonadism, small hands and feet, and mental retardation associated with characteristic behavior [49,50]. Approximately 70% of individuals with PWS have a deletion on the long arm of the paternal chromosome 15 [45,51], and most of those without a deletion of the paternal chromosome 15 have uniparental disomy for the maternal chromosome 15 [52,53].

Approximately 50% of individuals with PWS are hypopigmented but most do not have the typical ocular features of albinism [47–49]. Interestingly, a number of individuals with PWS and OCA have been identified [42]. For those without obvious OCA, hair and skin are lighter than unaffected family members, and childhood nystagmus and strabismus are common but often transient. The irides are pigmented with some translucency on globe transillumination, and retinal pigment is reduced in amount. Although foveal hypoplasia is not usually present, the fovea may not appear entirely normal [50]. Visual evoked potential studies have revealed optic tract misrouting similar to that found in albinism in some individuals with PWS and hypopigmentation [54]. This is an important observation because the presence of optic tract abnormalities implies pathologic hypopigmentation during development, thus indicating that the hypopigmentation in PWS is best described as a type of albinism. The individuals with PWS and OCA have not been fully described, but the features of the albinism are typical for OCA2.

Individuals with AS are also hypopigmented, but the percentage showing this phenotypic characteristic is unknown [42,48]. Angelman Syndrome is a complex developmental disorder that includes developmental delay and severe mental retardation, microcephaly, neonatal hypotonia, ataxic movements, and inappropriate laughter [55]. Those with hypopigmentation have light skin and hair and may have a history of nystagmus or strabismus [48]. Iris translucency and reduced retinal pigment may be present. No analysis of the optic tract organization is available.

PINK-EYED DILUTION GENE

The gene responsible for OCA2 has been identified as the human homologue to the mouse pink-eyed dilution (*p*) gene [39–42]. Two different deletions of the human P gene responsible for OCA2 have so far been identified in individuals with both PWS and OCA2. Southern blot analysis revealed that the maternally derived chromosome 15 contained a partial deletion of the P locus (the last exon of the P gene) and karyotype analysis demonstrated a *de novo* deletion of 15q11-q13 that included the entire P locus on the paternally derived chromosome [42]. A common 2.7-kb deletion of a single exon of the P gene has been identified in OCA2 individuals from the Brandywine isolate in Maryland, from unrelated African-American individuals, and from African individuals, showing that this mutation is common and has an African origin [44]. More importantly, the individuals in the Brandywine isolate were homozygous for this mutant allele, providing unequivocal evidence that mutations of the P gene are responsible for OCA2. Five different point mutations of the P gene associated with OCA2 have also been reported [43]. It has been hypothesized that the function of the P locus product may be a tyrosine transporter, and this would be consistent with the observation that fresh hairbulbs from individuals affected with OCA2 from melanosomal pigment when the hairbulbs are incubated in tyrosine (or DOPA). However, the reported similarity or the P protein sequence to a bacterial tyrosine transporter is weak. Studies are currently being carried out by several groups to investigate the function of this protein.

The mechanism for the hypopigmentation in PWS and AS in those individuals without obvious OCA has not been fully explained. The murine *p* locus is not imprinted and it is likely that the human locus is not [40]. The deletion on the paternal chromosome 15q in PWS and the maternal chromosome 15q in AS suggests that the hypopigmentation arises from a mechanism other than gene dose effect, because heterozygotes for OCA2 are normally pigmented. Further work will be necessary after the function of the gene product of the P locus is determined.

This investigation was supported by NIH grants GM22167 and AR40001. We would like to thank the generous participation of the families in this work. We would also like to thank Dr. Leonard Banaszak for help in computer modeling.

REFERENCES

1. King RA, Summers CG: Albinism. *Dermatol Clin* 6:217–288, 1988
2. King RA, Hearing VJ, Creel D, Oetting WS: Albinism. In: Scriver CR, Beaudet AL, Sly WS, Vallee D (eds.). *The Metabolic Basis of Inherited Diseases, 7th ed.* McGraw-Hill, New York, (in press)
3. Creel DJ, Witkop CJ, King RA: Asymmetric visually evoked potentials in human albinos: evidence for visual system anomalies. *Invest Ophthal* 13:430–440, 1974
4. Creel DJ, Summers CG, King RA: Visual anomalies associated with albinism. *Ophthalmic Paediatr Genet* 11:193–200, 1990
5. Nance WE, Jackson CE, Witkop CJ: Amish albinism: a distinctive autosomal recessive phenotype. *Am J Hum Genet* 22:579–586, 1970
6. Giebel LB, Tripathi RK, Strunk KM, Hanifin JM, Jackson CE, King RA, Spritz RA: Tyrosinase gene mutations associated with type IB ("Yellow") oculocutaneous albinism. *Am J Hum Genet* 48:1159–1167, 1991
7. King RA, Wirtschafter JD, Olds DP, Brumbaugh JA: Minimal pigment: a new type of oculocutaneous albinism. *Clin Genet* 29:42–50, 1986
8. King RA, Townsend D, Oetting WS, Summers CG, Olds DP, White JG, Spritz RA: Temperature-sensitive tyrosinase associated with peripheral pigmentation in oculocutaneous albinism. *J Clin Invest* 87:1046–1053, 1991
9. Giebel LB, Tripathi RK, King RA, Spritz RA: A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. *J Clin Invest* 87:1119–1122, 1991
10. Barton DE, Kwon BS, Francke U: Human tyrosinase gene, mapped to chromosome 11 (q14-q21), defines second region of homology with mouse chromosome 7. *Genomics* 3:17–24, 1988
11. Giebel LB, Strunk KM, King RA, Hanifin JM, Spritz RA: A frequent tyrosinase gene mutation in classic, tyrosinase-negative (type IA) oculocutaneous albinism. *Proc Natl Acad Sci* 87:3255–3258, 1990
12. King RA, Witkop CJ: Hairbulb tyrosinase activity in oculocutaneous albinism. *Nature* 263:69–71, 1976
13. King RA, Olds DP: Hairbulb tyrosinase activity in oculocutaneous albinism: suggestions for pathway control and block location. *Am J Med Gen* 20:49–55, 1985
14. Orita M, Suzuki Y, Sekiya T, Hayashi K: Rapid and sensitive detection of point

mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 5:874–879, 1989
15. Hayashi K: PCR-SSCP: a method for detection of mutations. *GATA* 9:73–79, 1992
16. Oetting WS, Fryer JP, Oofuji Y, Middendorf LR, Brumbaugh JA, Summers CG, King RA: Analysis of tyrosinase gene mutations using direct automated infrared fluorescence DNA sequencing of amplified exons. *Electrophoresis* 15:159–164, 1994
17. Oetting WS, King RA: Molecular basis of type I (tyrosinase-related) oculocutaneous albinism: mutations and polymorphisms of the human tyrosinase gene. *Hum Mut* 2:1–6, 1993
18. Oetting WS, Fryer JP, King RA: A dinucleotide deletion (−ΔGA115) in the tyrosinase gene responsible for type I–A (tyrosinase negative) oculocutaneous albinism in a Pakistani individual. *Hum Mol Genet* 7:1047–1048, 1993
19. Lerner AB, Fitzpatrick TB: Biochemistry of melanin formation. *Physiol Res* 30:91–126, 1950
20. Solomon EI: Coupled binuclear copper proteins: catalytic mechanisms and structure-reactivity correlations. In: King TE, Mason HS, Morrison M (eds.). *Oxidases and Related Redox Systems*. Alan R. Liss, New York, 1988, pp 309–329
21. Ross PK, Solomon EI: An electronic structural comparison of copper-peroxide complexes of relevance to hemocyanin and tyrosinase active sites. *J Am Chem Soc* 113:3246–3259, 1991
22. Körner A, Pawelek J: Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science* 217:1163–1165, 1982
23. Tripathi RK, Hearing VJ, Urabe K, Aroca P, Spritz RA: Mutational mapping of the catalytic activities of human tyrosinase. *J Biol Chem* 267:23707–23712, 1992
24. Oetting WS, King RA: Molecular analysis of type I-A (tyrosinase negative) oculocutaneous albinism. *Hum Genet* 90:258–262, 1992
25. Oetting WS, King RA: Analysis of mutations in the copper B binding region associated with type I (tyrosinase related) oculocutaneous albinism. *Pig Cell Res* 5:274–278, 1992
26. Lerch K: Neurospora tyrosinase: structural, spectroscopic and catalytic properties. *Mol Cel Biochem* 52:125–138, 1983
27. Huber M, Lerch K: Identification of two histidines as copper ligands in *Streptomyces glaucescens* tyrosinase. *Biochemistry* 27:5610–5615, 1988
28. Jackman MP, Hajnal A, Lerch K: Albino mutants of *Streptomyces glaucescens* tyrosinase. *Biochem J* 274:707–713, 1991
29. Müller G, Ruppert S, Schmid E, Schütz G: Functional analysis of alternatively spliced tyrosinase gene transcripts. *EMBO J* 7:2723–2730, 1988
30. Gaykema WPJ, Hol WGJ, Vereijken JM, Soeter NM, Bak HJ, Beintema JJ: 3.2 Å structure of the copper-containing, oxygen-carrying protein *Panulirus interruptus* haemocyanin. *Nature* 309:23–29, 1984
31. Gaykema WPJ, Volbeda A, Hol WGJ: Structure determination of *Panulirus interruptus* haemocyanin at 3.2 Å resolution: successful phase extension by six-fold density averaging. *J Mol Biol* 187:255–275, 1985
32. Jackman MP, Huber M, Hajnal A, Lerch K: Stabilization of the oxy form of tyrosinase by a single conservative amino acid substitution. *Biochem J* 282:915–918, 1992
33. Kraulis PJ: MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J Appl Crystal* 24:946–950, 1991
34. Witkop CJ, Hill CW, Desnick S, Thies JK, Thorn HL, Jenkins M, White JG: Ophthalmologic, biochemical, platelet and ultrastructural defects in the various types of oculocutaneous albinism. *J Invest Dermatol* 60:443–456, 1973
35. Nance WE, Witkop CJ, Rawls RF: Genetic and biochemical evidence for two forms of oculocutaneous albinism in man. *Birth Defects: Original Article Series* 7:125–128, 1971
36. Witkop CJ, Niswander JD, Bergsma DR, Workman PL, White JG: Tyrosinase positive oculocutaneous albinism among the Zuni and Brandywine triracial isolate: biochemical and clinical characteristics and fertility. *Am J Phys Anthrop* 36:397–405, 1972
37. King RA, Olds DP: Electrophoretic pattern of human hairbulb tyrosinase. *J Invest Dermatol* 77:201–204, 1981
38. King RA, Olds DP, Witkop CJ: Characterization of human hairbulb tyrosinase: properties of normal and albino enzyme. *J Invest Dermatol* 71:136–139, 1978
39. Nakatsu Y, Gondo Y, Brilliant M: The *p* locus is closely linked to the mouse homologue of a gene from the Prader-Willi chromosomal region. *Mam Genome* 2:69–71, 1992
40. Gardner JM, Nakatsu Y, Gondo Y, Lee S, Lyon MF, King RA, Brilliant MH: The mouse pink-eyed dilution gene: association with human Prader-Willi and Angelman syndrome. *Science* 257:1121–1124, 1992
41. Ramsay M, Colman MA, Stevens G, Zwane E, Kromberg J, Farrall M, Jenkins T: The tyrosinase-positive oculocutaneous albinism locus maps to chromosome 15q11.2-q12. *Am J Hum Genet* 51:879–884, 1992
42. Rinchik EM, Bultman SJ, Horsthemke B, Lee S-T, Strunk K, Spritz RA, Avidano KM, Jong MTC, Nicholls RD: A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. *Nature* 361:72–76, 1993
43. Lee S-T, Nicholls RD, Bundey S, Laxova R, Musarella M, Spritz RA: Mutations of the P gene in oculocutaneous albinism, ocular albinism and Prader-Willi syndrome plus albinism. *N Engl J Med* 330:529–534, 1994
44. Durham-Pierre D, Gardner JM, Nakatsu Y, King RA, Franke U, Ching A, Aquaron R, del Marmol V, Brilliant MH: African origin of a common mutation of the human P gene in African American tyrosinase positive oculocutaneous albinism (OCA2). *Nature Genetics* 7:176–179, 1994
45. Butler MG, Meaney FJ, Palmer CG: Clinical and cytogenetic survey of 39 individuals with Prader-Labhart–Willi syndrome. *Am J Med Genet* 23:793–809, 1986
46. Wiesner GL, Bendel CM, Olds DP, White JG, Arthur DC, Ball DW, King RA: Hypopigmentation in the Prader-Willi syndrome. *Am J Hum Genet* 40:431–442, 1987
47. Butler MG: Hypopigmentation: a common feature of Prader-Labhart-Willi syndrome. *Am J Hum Genet* 45:140–146, 1989
48. King RA, Weisner GL, Townsend D, White JG: Hypopigmentation in Angelman syndrome. *Am J Med Genet* 46:40–44, 1993
49. Cassidy SB: Prader-Willi syndrome. *Curr Prob Pediat* 14:5–55, 1984
50. Bray GA, Dahms WT, Swerdloff RS, Fiser RH, Atkinson RL, Carrel RE: The Prader-Willi syndrome: a study of 40 patients and a review of the literature. *Medicine* 62:59–80, 1983
51. Ledbetter DH, Mascarello JT, Riccardi VM, Harper VD, Airhart SD, Strobel RJ: Chromosome 15 abnormalities and the Prader-Willi syndrome: a follow-up report of 40 cases. *Am J Hum Genet* 34:278–285, 1982
52. Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown MG, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J, Lacy D, LaFranchi S, Lahr M, Macfarlane J, Williams CPS: Comparison of the 15q deletions in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin, and clinical consequences. *Am J Med Genet* 35:333–349, 1990
53. Mascari MJ, Gottlieb W, Rogan PK, Butler MG, Waller DA, Armour AL, Jeffreys AJ, Ladda RL, Nicholls RD: The frequencies of uniparental disomy in Prader-Willi syndrome: implications for molecular diagnosis. *N Engl J Med* 326:1599–1607, 1992
54. Creel DJ, Bendel CM, Wiesner GL, Wirtschafter JD, Arthur DC, King RA: Abnormalities of the central visual pathways in Prader-Willi Syndrome associated with hypopigmentation. *N Engl J Med* 314:1606–1609, 1986
55. Fryburg JS, Breg WR, Lindgren V: Diagnosis of Angelman syndrome in infants. *Am J Hum Genet* 38:58–64, 1991